Can different crop rotation management affect N_2O emissions? An incubation experiment using uruguayan soils from a long-term study conducted within a denitrification system at Rothamsted Research, North Wyke.

Mariana Rosas Alenicov

INIA Uruguay/ University of The Republic, Uruguay

mralenicov@gmail.com

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Host institution and contact:

Rothamsted Research, North Wyke, Devon

Department: Net Zero and Resilient Farming

Dra. Laura Cárdenas, laura.cardenas@rothamsted.ac.uk

Abstract

Greenhouse gas emissions (GHG) from the agricultural sector are a major concern. One of the most potent GHG is Nitrous Oxide (N_20), which around 60% of the emissions comes from the agricultural soils. The N_20 production depends on many variables, such us WFPS, temperature, pH, management, Carbon and Nitrogen content. In this experiment we are going to study the effect of soil management, the dynamic and interaction between C and N in the N_2O emissions from soils of a long-term experiment of Uruguay. Soils has been 60 years under different managements, for the study we select (from 7 treatments) 2 rotations: 1- CA continuous agriculture, from a typical crop rotation in Uruguay; and 2-CP the same crop rotation with 50 % of the time with pasture. Soils were sampled in June 2024 and sent to the UK to do an incubation in the Denitrification system at Rothamsted Research in Devon. To study the effect of the different carbon content because of different crop rotation in the emissions.

Keywords: Greenhouse gas, emissions, agriculture, N_2O, crop rotation.

Context

It is a worldwide issue the adaptation and mitigation of the global warming effect due to the increment in greenhouse gas emissions (GHG). The agricultural sector has been working in developing and implementing practices and managements to limit or reduce emissions that result from the sector activities, mainly focused on methane (CH4) and nitrous oxide (N2O).

Nowadays, there are also several efforts promoting soil carbon sequestration in order to compensate for GHG emissions and to increase the stock of organic carbon in soils (SOC). However, as the cycle of carbon is very related with the dynamic of nitrogen in soils, it is very important to study this interaction for different type of soils (Guenet et al., 2021). The importance of studying the nitrogen cycle, lies in the fact that in one hand it is the precursor of an important GHG (N2O), and on the other hand, most of N2O emissions comes from the soils (Signor & Cerri, 2013). A lot of literature is available whereas applying practices to improve SOC stocks, may lead to an increase or a decrease of N2O emissions (Guenet et al., 2021).

Emissions of N2O from soils result from four main processes: nitrification, denitrification, chemidenitrification and hydroxylamine oxidation. The first two are biological processes and are the most relevant in terms of N2O production in soils, while the other two are chemical processes. Therefore, any factor that affects microorganisms' lives or activity in soils also affects the production and emission of N2O. This highlights the importance of understanding the conditions under which these factors influence microorganisms and, consequently, N_2O production.

There are many factors that affect the processes mentioned above that define N2O emissions from soils. Soil conditions such as O2 concentration, temperature, % of humidity, pH, % of Organic Matter (OM), % of Nitrogen (N), Carbon/Nitrogen ratio (C/N), and texture (Signor & Cerri, 2013) are within the main factors affecting N2O. At the same time, many of those factors are largely affected by the agricultural

systems conducted including the crop and pasture rotation, and the soil management (e.g. till, reduced till, no till, etc).

In Uruguay, a long-term experiment was installed in 1963 on agricultural soils to assess the sustainability of crop rotations and sequences under no-till farming, with and without pastures. This experiment represents typical crop-pasture rotation systems of the country and offers the opportunity to study and understand locally the soil carbon-nitrogen relationship and identify the contributions of each system for GHG mitigation.

Hypothesis

The hypothesis is that a soil with different management and crop rotation for 60 years presents different soil organic carbon and emits different rates of N2O.

Objectives and main purpose of fellowship

The objective of this study was to study and quantify the long-term effects of crop-pasture rotation systems on N_2O, N_2, and CO_2 emissions from an uruguayan agricultural soil.

The main objective of my internship at Rothamsted Research in Devon, UK was to run an incubation experiment at the Denis Laboratory, to study the soil N_2O, N_2, and CO_2 emissions from two contrasting agricultural management systems from a long-term study of Uruguay. In Rothamsted Research, Devon, there is a DENIS Laboratory which is a denitrification incubation system, with enclosed 12 vessels flow for incubating soil cores and continuously measured emissions such as N_2O, N_2, CO_2, NO. The innovation of this system is that it is a free N_2 environment, with temperature control. This system is very important to study denitrification process and has the possibility to add amendment without disturbing the controlled atmosphere (Cárdenas et al., 2003).

Materials and methods

Soil sampling and preparation

The soils were sampled from the long-term experiment “José Lavalleja Castro” in La Estanzuela, at the research institute National Institute of Agricultural Research “INIA”, from its Spanish acronym, in Uruguay (34°20'34.6"S 57°43'26.5"W), as is shown in figure 1.

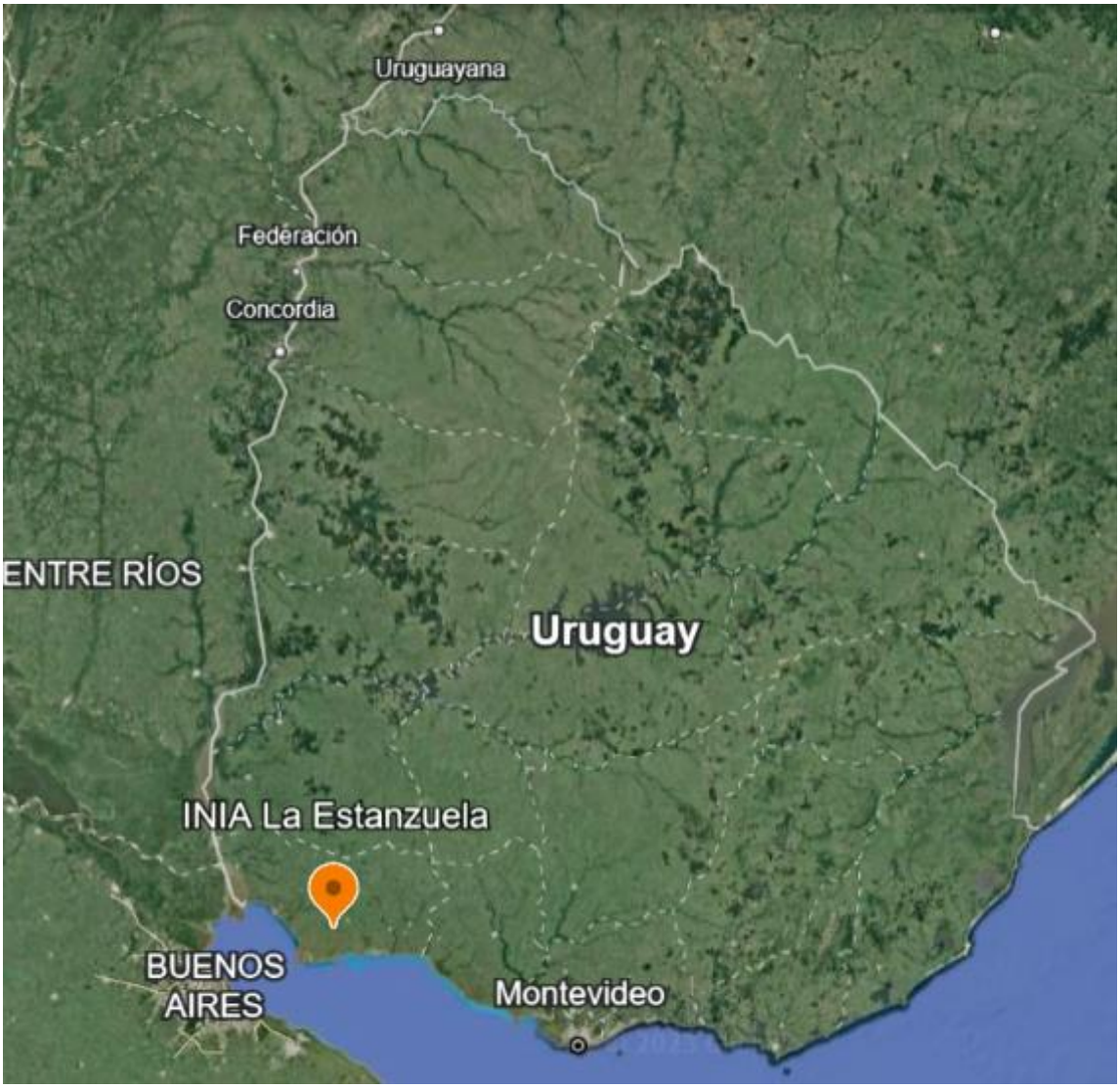


Figure 1: Experiment site in Uruguay. Image taken from Google Earth.

The experiment design consists of seven treatments, each with a rotation sequence of six years, organized in a randomized complete block design with three replications. In order to take into account, the year effect (i.e. climate effects) treatment repetitions follow a staggered-start design. Plots are 25 meters by 200 meters long, with 2.5–3% slope. Soil is Vertic Argiudoll, based on USDA Soil Taxonomy, (Soil Survey Staff, 2010), considered as a silty clay loam texture class (Rubio et al., 2021). This experiment was established in 1963, and the main objective is to evaluate the effect of the crop rotation, with different components of grassland (from 0 to 66 % of the time), on soil properties, crop yield and environment.

For this study, two treatments were selected: Continuous Agriculture (CA) and Crop rotation with pastures 50 % of the sequence (CP). Soils samples were taken in July 2024 from each of the three plots (repetitions) from the two treatments, using a Zig-Zag method to capture the variability in the space, with a soil sampler of 0- 15 centimeters depth. Due to the staggered design, each plot was in a different stage of the sequence (Figure 2).

Continuous agriculture			Crop pasture 50%		
Plot 5	**Plot 9**	**Plot 18**	**Plot 6**	**Plot 8**	**Plot 20**
Wheat	Barley	Maize	Pasture - year 3	Pasture - year 2	Pasture- year 1

Figure 2: Lan use in plot sampled

Gravimetric water content (GWC) was 26% after sampling. The soil was packaged in a double plastic bag and sent to Rothamsted Research. In preparation to package the samples into cores for the incubation, they were previously sieved to 2 mm.

Two different types of cores were prepared for the entire experiment: P-Denis cores and Parallel cores. The first ones were stainless steel of 10 cm height and 13 cm diameter (Figure 3). These cores were inserted into the chambers in the incubation system. Parallel cores were plastic of 10 cm height and 4,5 diameter (Figure 4). These cores were used in a parallel incubation in an environment room with the same treatment of the P-DENIS cores (temperature, nitrogen and %WFPS), and were used to do destructive analysis (more details in experiment details).

To generate the best situation to promote denitrification, we define 75 % WFPS, amendment of 125 Kg of Nitrogen per hectare, and temperature was at 20 Celsius degrees (average soil temperature in Uruguay 17 C), based on the literature review (Cárdenas et al., 2003), (Loick et al., 2021), this conditions were stablished in all the chambers.

Both cores were packed with a bulk density of 1,25 g /cm3 to simulate the natural bulk density (BD) of soils BD 1,33 g/cm3.

Figure 3:Soil packaging in P-Denis cores.

Figure 4: Soil packaging in parallel soil incubation

Incubation system

The experiment was conducted at P-DENIS laboratory, which is a specialized incubation system that allows to study denitrification from soils in detail, since the N_2 is removed from the atmosphere, replacing it with a mixed mixture of Helium (He) and Oxygen (O_2). This is the innovation of the system, as it allows for the measurement of N_2O produced from the nitrogen content in soils, after eliminating N_2 from the atmosphere.

P-DENIS system has 12 cores, so the treatment were disposed as it shows in figure 5, being CH-1 to CH-12 is the number of the chambers in the system, inside each circle is indicated the treatment, where CA or CP is the treatment and after the hyphen the crop or pasture: Wheat (W), Barley (B), Maize (M), Pasture first year (P1), Pasture second year (P2), pasture third year (P3). Figure 6 shows the experiment with the chambers ready to start.

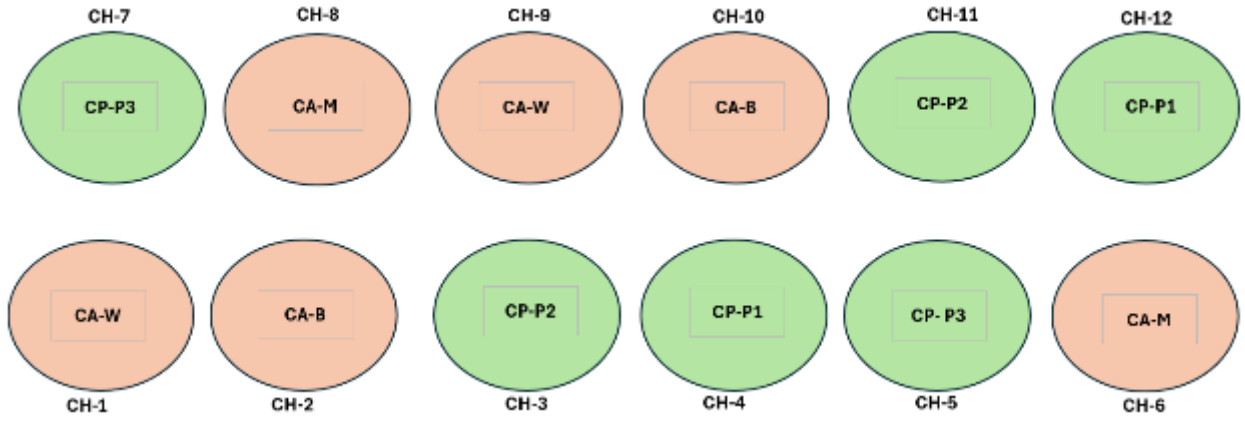


Figure 5: Schematic diagram of the treatments in chambers at P-Denis system.

Figure 6: Chambers set up at P-Denis system.

Experimental details

The experiment calendar and agenda of the incubation is presented in Table 1. In the table "Time" means the days before (with minus) or after the amendment, day 0 is the starting day of experiment, defined by the fertilization with KNO^-_3. In order to maintain 75% WFPS in parallel incubation cores, daily water correction was required (Figure 7).

A parallel incubation was conducted at the same time as the incubation, with the same packaging conditions BD, %WFPS, dose of Nitrogen amendment and temperature. The samples were all the time while running the incubation in an "environment room" and a tiny tag was set up to measure the temperature during the incubation period. The samples in parallel cores were used for destructive analysis to determine pH, moisture content, TON, NH4, %C in three times:

Time 0: The day of the fertilization (in parallel cores was three days after the fertilization in P-Denis system)

Time 1: Three days after the N_2O peak started to decrease.

Time 2: Three days after the incubation stopped.

Table 1: Experiment calendar and details of activities

Date	Time	Coment
22/10/2024	-10	Packing P-Denis and parallel cores
24/10/2024	-8	Setting up the system (temperature, connecting lines). Tiny tag in P-Denis system. Start flushing with 168ml/min (He 80%-O_2 20%)
25/10/2024	-7	Analyze flows, and N_2 background. Set up tiny tag in environment room
26/10/2024	-6	Change drying tubes. System stopped because N2 levels were too high. Checked for system losses
27/10/2024	-5	Some lines were blocked, so decided to raise the flushing to clean the system to 300ml/min (He 80%-O_2 20%)
28/10/2024	-4	Decreased flow to 168 ml/min
29/10/2024	-3	System stopped because N_2 levels still very high. Increased system flow to 360 ml/min (He 80%-O_2 20%)
30/10/2024	-2	Chambers 1, 3, 5, 6, 8 and 11 were blocked.
31/10/2024	-1	Resumed analysis with 8 chambers. Chambers 3, 5, 8 y 11 were removed because of blockages in system lines.
1/11/2024	0	The drying tubes were changed. CH1 got blocked. Flow changed to 98 ml/min, being 14 mil/min per chamber. KNO3 fertilization was done. Dose: 125 Kg N/ha. Time 0, destructive analysis of parallel incubation to analyze, pH, GWC% TON, NH_4 and % C.

2/11/2024	1	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
3/11/2024	2	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N2O and CO_2 integration curve.
4/11/2024	3	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve. Fertilization with KNO3 of parallel incubation, Same dose of P-Denis cores (125 kg N/Ha)
5/11/2024	4	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
6/11/2024	5	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
7/11/2024	6	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
8/11/2024	7	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve. N2O peak start going down
9/11/2024	8	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
10/11/2024	9	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
11/11/2024	10	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve. Time 1, destructive analysis of parallel incubation cores to determine: pH, GWC% TON, NH4 and % C.
12/11/2024	11	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N2O and CO_2 integration curve.
13/11/2024	12	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N2O and CO_2 integration curve.
14/11/2024	13	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
15/11/2024	14	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.

16/11/2024	15	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
17/11/2024	16	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
18/11/2024	17	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
19/11/2024	18	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
20/11/2024	19	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
21/11/2024	20	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
22/11/2024	21	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
23/11/2024	22	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
24/11/2024	23	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
25/11/2024	24	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
26/11/2024	25	Daily activities: change drying tubes, water correction in parallel incubation, Check chambers flows, check N_2O and CO_2 integration curve.
27/11/2024	26	Stopped the system. P-Denis cores to analyse: pH, GWC% TON, NH4 and % C. Clean the system.
28/11/2024	27	Clean the laboratory, update information on the computer. Process samples to determine pH, %C and moisture
29/11/2024	28	Clean the laboratory, update information on the computer. Process samples to determine pH, %C and moisture
30/11/2024	29	Clean the laboratory, update information on the computer. Process samples to determine pH, %C and moisture
1/12/2024	30	Time 2, destructive analysis of parallel incubation cores to determine: pH, GWC% TON, NH_4 and % C.
2/12/2024	31	Start analysing data

Figure 7: Daily weight control in parallel cores.

Determinations of NO_3^- and NH_4^+ were done using the KCL extraction technique provided by Rothamsted Research laboratory (Figure 8). Soil pH determination was done in water (Figure 9).

Figure 8: KCL soil extraction to determine nitrate and ammonium.

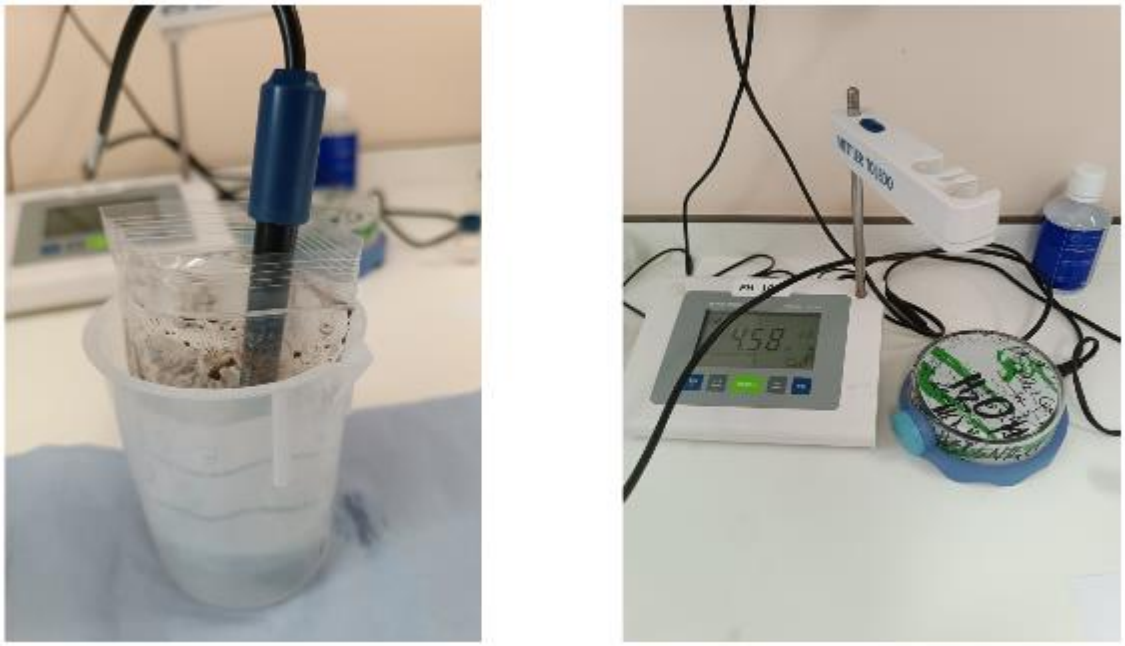
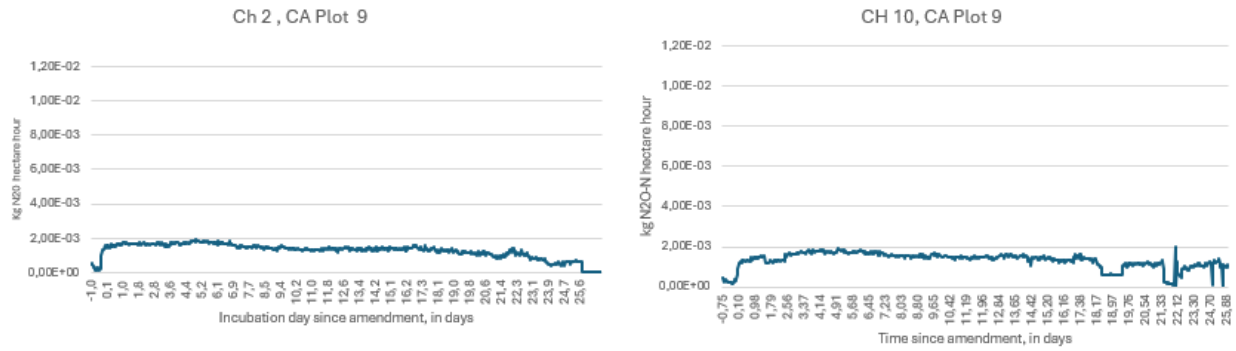


Figure 9: pH determination in water.

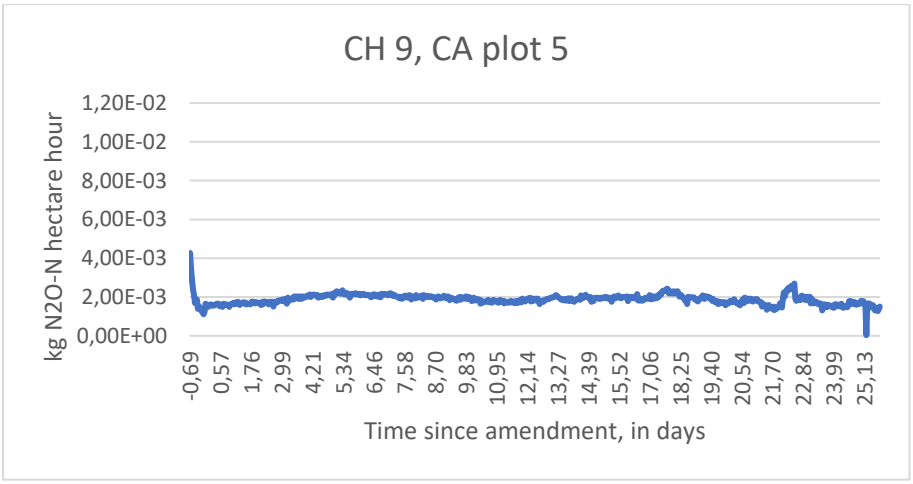
Preliminary results

It is important to notice that nowadays we are still analysing the data obtained from the experiment, but the information shared in the next graphics is part of the preliminary results from the gases analysis. At the beginning of the experiment, some gas lines were blocked; therefore, we couldn't measure the emissions from chamber 1, 3, 5, 8 and 11, and we lost value information. In consequence some chambers do not have any repetition.

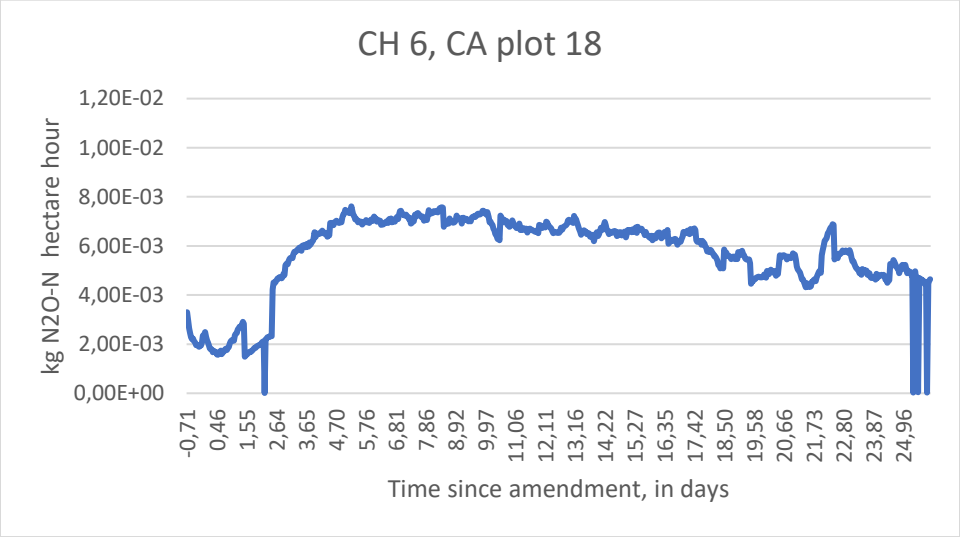
From graphics 1 to 6, we can observe the kg of N_2O-N emitted to the atmosphere from each treatment, expressed per hectare and per hour. Although the results show a trend effect on N_2O emissions from each treatment, it is not possible yet to extract research conclusions. Nevertheless, these primary results are already indicating the need of a new incubation experiment that could answer new research questions.



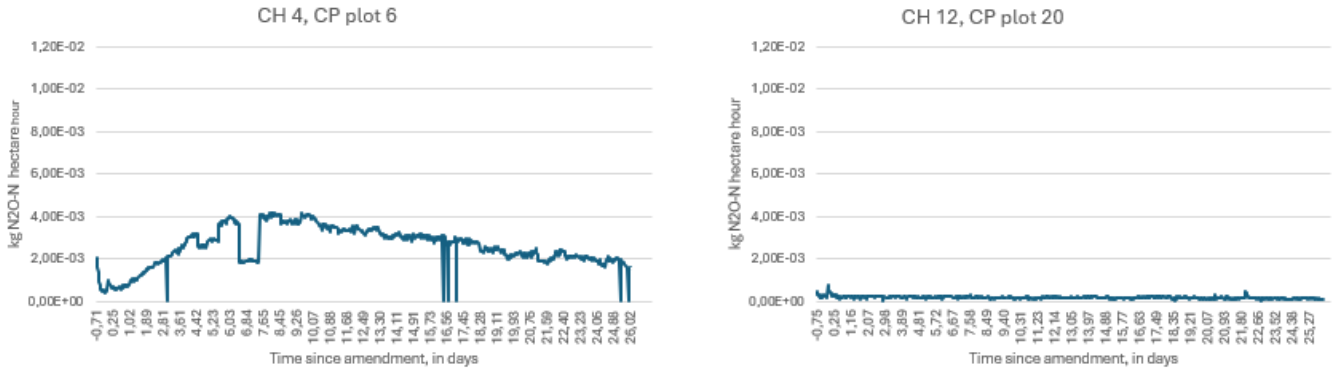
Graphic 1: kg N_2O -N per hectare per hour emissions for Continuous agriculture.



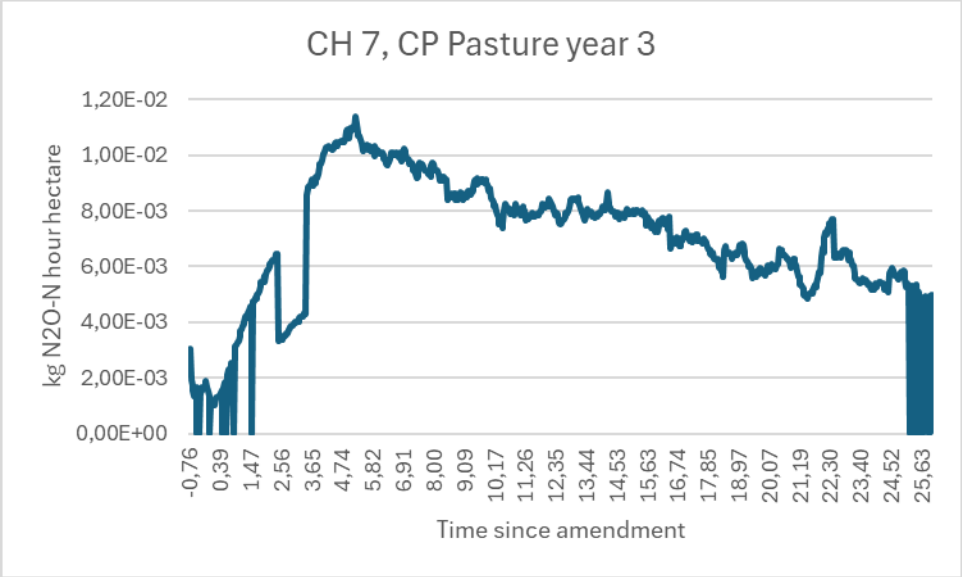
Graphic 2: kg N_2O -N per hectare per hour emissions for Continuous agriculture.



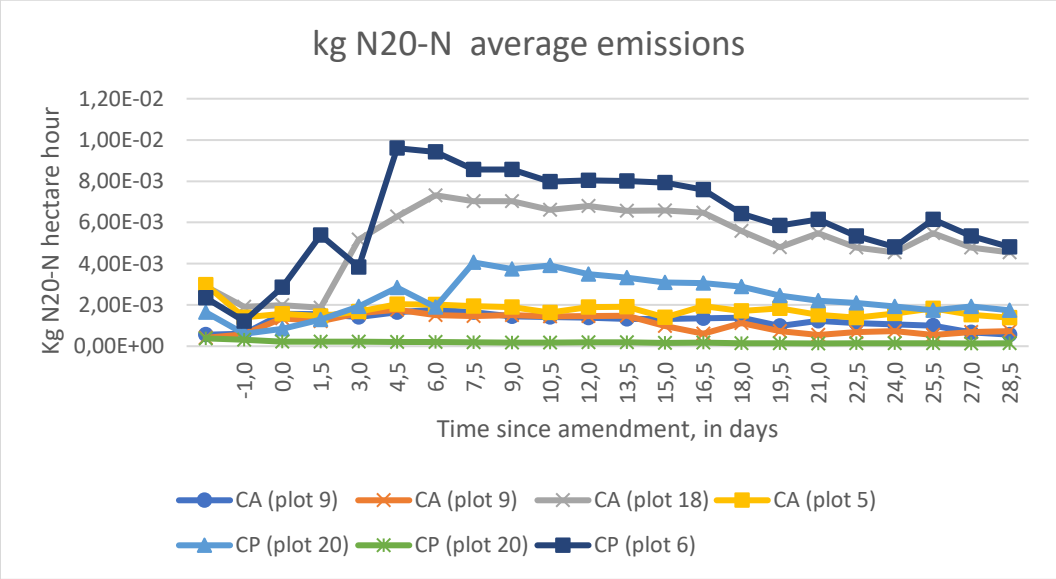
Graphic 3: kg N_2O -N per hectare per hour emissions for Continuous agriculture.



Graphic 4: kg N_2O -N per hectare per hour emissions for Crop pasture rotation.

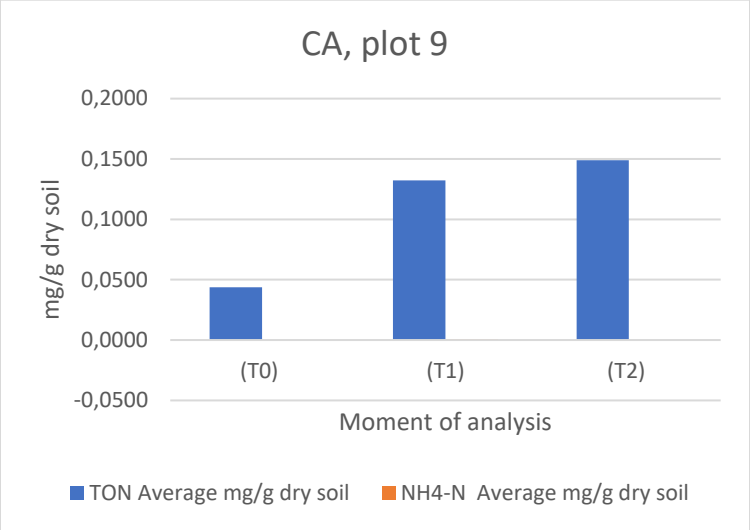


Graphic 5: kg N2O -N per hectare per hour emissions for Crop pasture rotation.

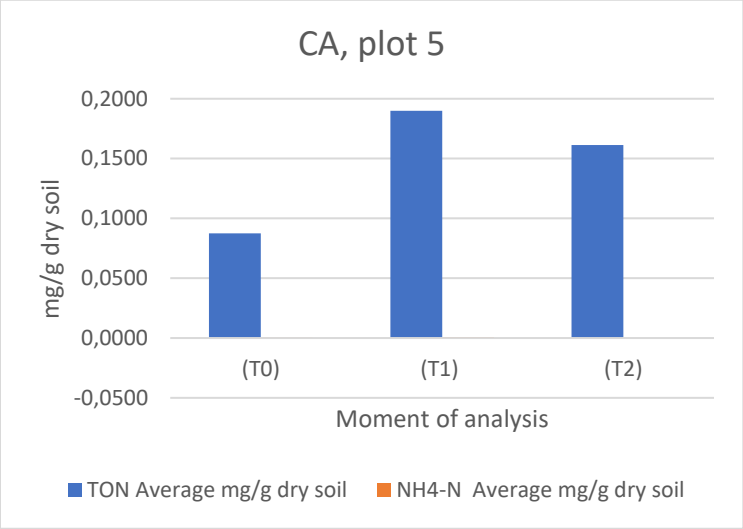


Graphic 6: Kg N_2O -N per hectare per hour on average per day of incubation for the different treatments.

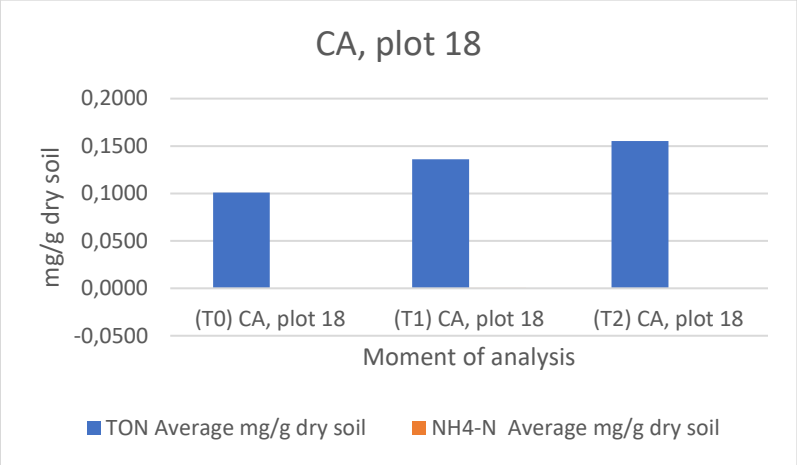
The data below shows the (TON) NO^-_3 and NH^+_4 in mg/ g of dry soil from parallel incubation (explained before). We indicate the treatment and time of the sample. Where (T0), (T1) and (T2) are the moments of the sample time: day of the amendment, three days after the peak of N_2O starts going down, and three days after the end of the experiment, respectively. We are still processing the results from organic C and N from these samples, once we have all the information regarding soils, we are going to be able to discuss the results.



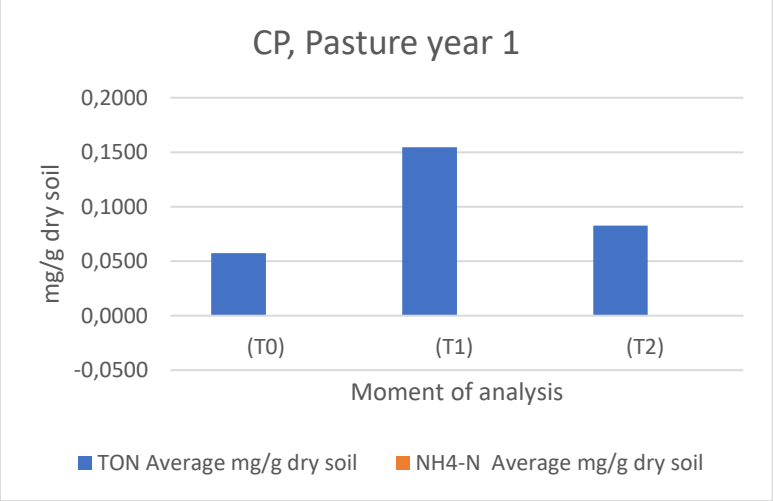
Graphic 7: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for AC, with barley as crop at the moment of soil sample.



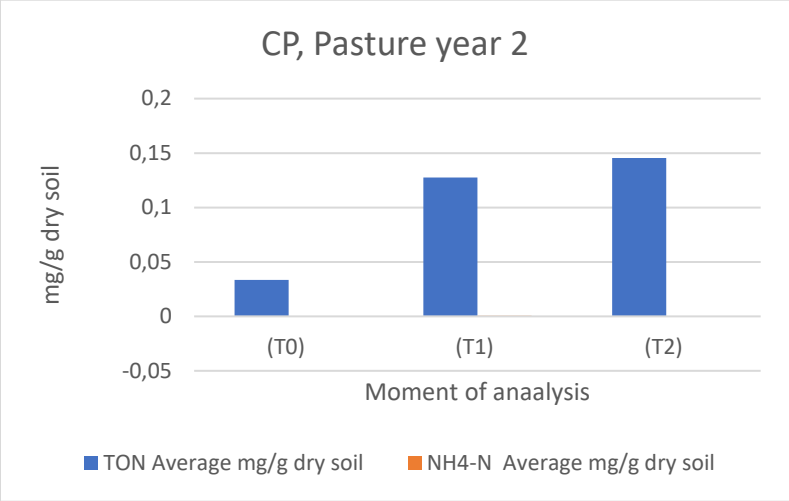
Graphic 8: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for AC, with wheat as crop at the moment of soil sample.



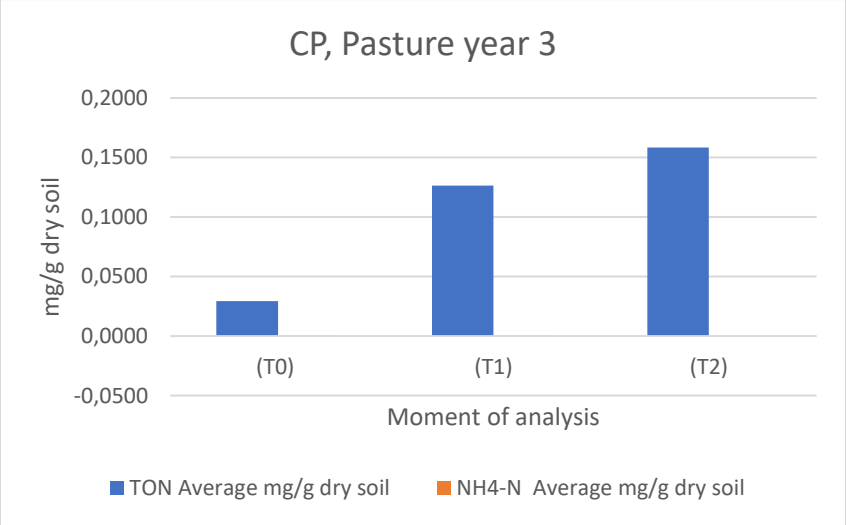
Graphic 9: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for AC, with maize as crop at the moment of soil sample.



Graphic 10: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for CP, with first year of pasture implementation at the moment of soil sample.



Graphic 11: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for CP, with second year of pasture implementation at the moment of soil sample.



Graphic 12: TON and NH4 in mg-g dry soil in T0, T1 AND T2 for CP, with third year of pasture implementation at the moment of soil sample.

Other activities involved

During the internship, I had the opportunity to meet researchers from different groups that allows me to get involved in other activities while the incubation was running.

Determination of 15N in Nitrate and Ammonium from N_2O production:

I have learnt different laboratory techniques while I have got involved in a soil incubation experiment with isotopes signals. To read the isotopic signals from the enrichment of the $N0_3$ and NH_4, first a transformation to N_2O is required, since the IRMS read the enrichment in the gas phase. This is a very complex technique which requires various steps and many days. Some pictures of the process are shared in the figures below:

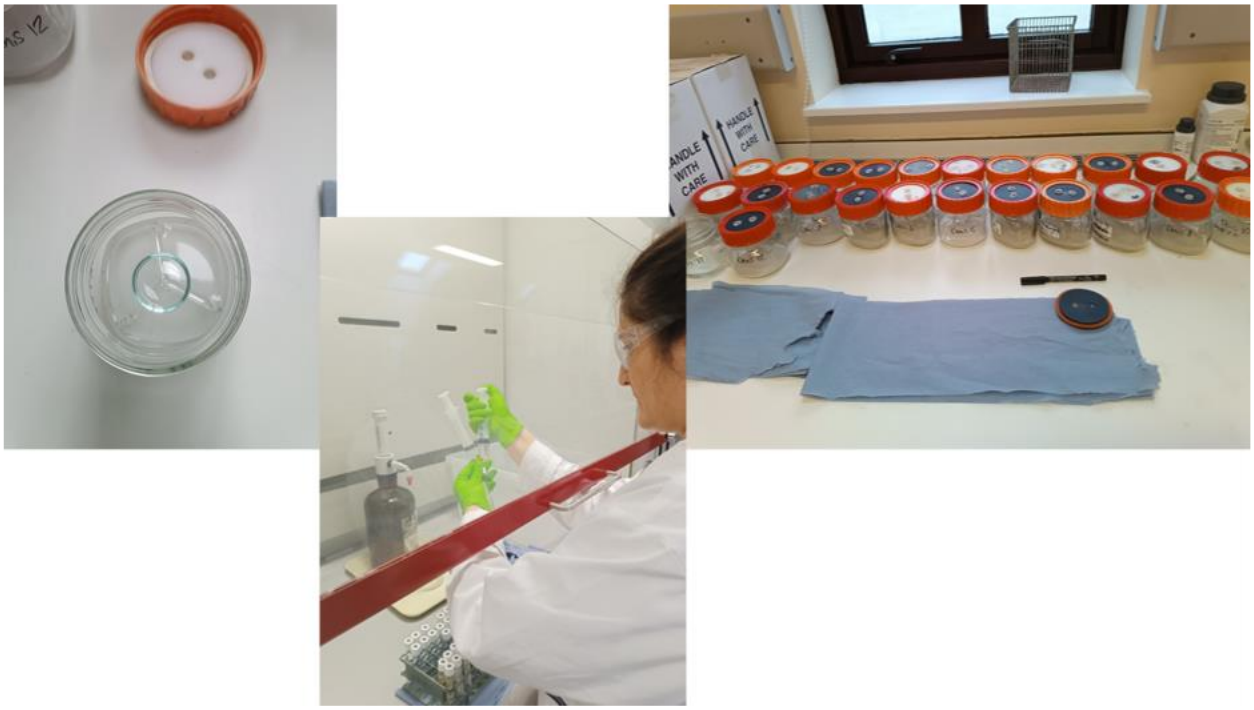


Figure 10: N_2O production from NH^+_4.

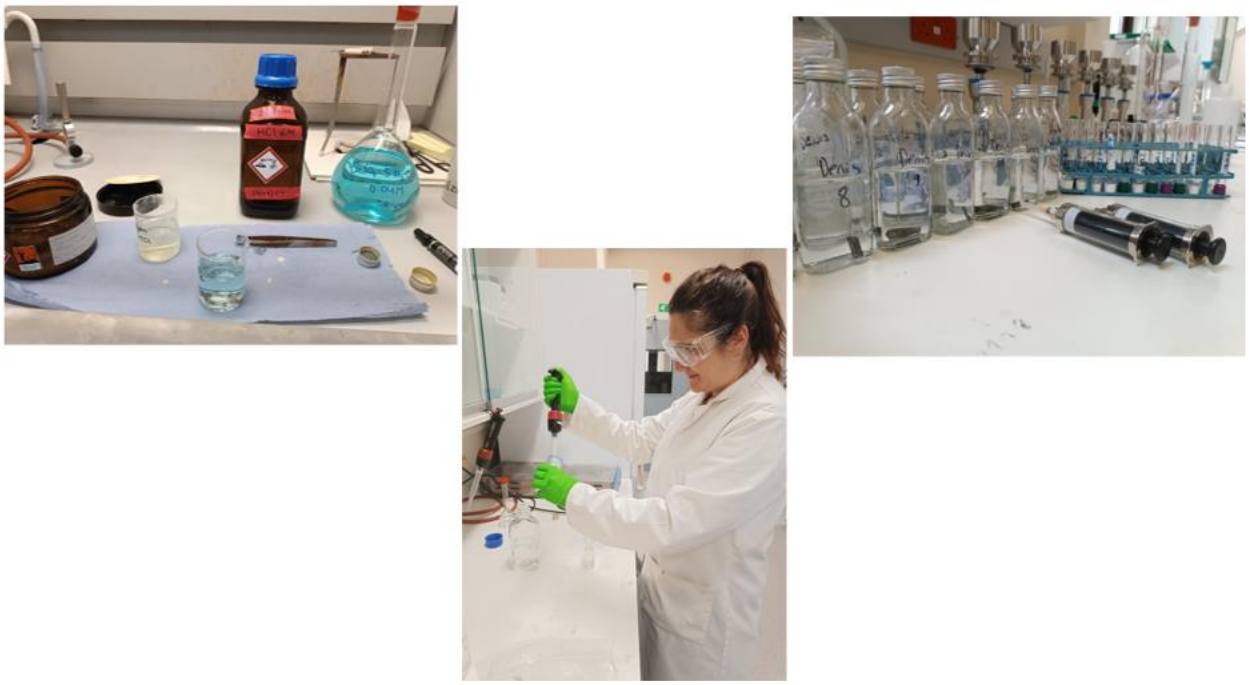


Figure 11: N_2O production from NO^-_3.

Soil sampling in agroforestry farm at Wakelyns:

I had the opportunity to join a group of researchers to go to a farm with agroforestry production, this is a very innovating system running since 1994. The farm located in Fressingfield welcome the work group of Rothamsted to take samples from different areas (all related to soil) to study the long-term effect of implementing an agroforestry production system in soil structure, soils health, GHG, chemical and physical properties, and the impact on microorganisms.

Figure 12: Part of the work group at the agroforestry farm.

Participation inf the First science day at Rothamsted Research:

During my stay at Rothamsted Research, I had the great pleasure to attend to the first science day organized by my tutor Laura Cardenas about "**Atmospheric Emissions science"**. This is the first of many, in which every area presents the experiments on going, results and future investigations lines of work. It was a full day agenda with seminars, lunch break with poster exhibitions and farm visits. This was a very good opportunity for me, because I learnt a lot about the research experiments, the technology implemented and met a lot of great people.

Figure 13: First Science Day "Atmospheric Emissions science"

Experience gained

During the internship at Rothamsted, I have learnt many different things, involving theory, practical, laboratory techniques, cultural and personal development. I had the opportunity to learn laboratory techniques from simple ones such us pH determination in water, to more specialized ones for example Nitrate and ammonium transformation to N_2O . I went to help in a soil sampling activity, and during this work expedition I met the countryside and learn about other sustainable productions system, at the same time I could learn from the researchers from different samples techniques and soils about UK.

Other experienced gained, from a more personal point of view is the cultural exchange, I lived and share time with other student from different cultures, which they taught me a lot from their countries. Last, but not least, this experience helped me to get involved with many different investigators.

Future development

This internship provides me a lot of future lines of investigations, besides this are preliminary results, we already discuss new hypothesis with the group in Rothamsted Research and INIA Uruguay, so in the upcoming months a second incubation with new objectives. One objective from this visiting is the publication of an article in a scientific journal in collaboration with my tutors in Uruguay and UK.

Acknowledgments

I find it challenging to express my gratitude without leaving someone out or failing to acknowledge everyone who has helped along the way. However, I will start by first thanking the entire team at the Stapledon Memorial Trust for trusting in my research proposal and funding my internship. Undoubtedly, this experience marks a significant step in my professional career and will open many doors for me in the future. I will be eternally grateful, for the support provided, both for my professional development and personal growth.

My sincere thanks go to my tutor and mentor in Uruguay, Verónica Ciganda, who believed in me and offered the opportunity to represent the institution abroad—certainly one of the most important challenges I have ever faced.

My deepest gratitude extends to Laura Cardenas, my tutor in the UK, for her invitation and warm welcome. Her support, kindness, and patience made my stay at Rothamsted Research a beautiful experience.

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